center stage in orchestrating multiple events during chromosomal replication.

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Chromosome Congression: The Kinesin-8-Step Path to Alignment

During mitosis, chromosomes must become aligned at the equator of the mitotic spindle before segregation. Recent work suggests that a kinesin-8 motor uses a unique combination of activities to regulate this process.

Jason Stumpff and Linda Wordeman

To segregate their chromosomes properly during cell division, eukaryotic cells employ a system of proteins to capture, bi-orient and position chromosomes at the equator of the microtubule-based mitotic spindle. Chromosomes make relatively stable attachments to spindle microtubule plus-ends through specialized protein complexes called kinetochores. Throughout the process of chromosome alignment, referred to as 'congression', kinetochore microtubules elongate and shorten while maintaining attachment to the chromosomes. Detailed observations of vertebrate chromosome movements and micromanipulation studies have established that assembly (lengthening) and disassembly (shortening) of the 20–25 microtubules that bind each vertebrate kinetochore may contribute the bulk of the forces required for chromosome congression during early mitosis, as well as chromosome segregation during anaphase [1]. A paper published recently in *Current Biology* [2] suggests that the human kinesin-8 motor, Kif18A, regulates kinetochore microtubule dynamics to promote chromosome congression.

Chromosomes attached to kinetochore microtubules in vertebrate cells typically move with a constant velocity (1–3 μm per min) and make rapid directional changes from poleward to anti-poleward movement, where the direction of movement is described relative to the spindle pole to which the chromosome is attached. The direction of chromosome movement is complemented by kinetochore microtubule plus-end dynamics. The addition of αβ-tubulin dimers at kinetochore microtubule plus-ends correlates with anti-poleward movements and removal of tubulin dimers results

in poleward movements. Once chromosomes reach the equator of the spindle, they continue to undergo oscillations of alternating poleward and anti-poleward movements until anaphase onset [1]. Interestingly, the sole parameter of movement that changes after chromosome alignment is the microtubule 'switching' potential: the potential of a microtubule to switch from assembly to disassembly (catastrophe) or vice versa (rescue). The switching potential is lower before alignment, resulting in directionally persistent chromosome movements [3]. Modeling studies in yeast have implicated a spatial gradient of either microtubule catastrophe or rescue as an essential component of chromosome positioning [4].

Molecules that modulate microtubule dynamics, especially with respect to switching parameters, are likely candidates to promote chromosome congression. Previous studies have implicated the kinesin-8 motors in chromosome congression; however, the specific contribution that these motors make to chromosome movements remains unsolved [5–10]. The new work of Mayr *et al.* [2] suggests that a human kinesin-8 motor, Kif18A, may use a combination of directional motility and microtubule depolymerization to regulate microtubule dynamics and control chromosome movements during mitosis [2]. The authors showed that Kif18A depolymerizes stable microtubules *in vitro* and localizes to the plus-ends of kinetochore microtubules in mitotic cells. Depletion of Kif18A was found to affect kinetochore movements and result in mitotic cells with abnormally long spindles that fail to congress chromosomes (Figure 1A).

Interestingly, Kif18A, like its yeast ortholog Kip3p, translocates unidirectionally along microtubules and depolymerizes stable microtubule plus-ends *in vitro* [2,11,12]. Similar to the depolymerizing kinesin-like motors of the kinesin-13 family, the ATPase activity of kinesin-8 motors is stimulated by both microtubules and free-tubulin, suggesting that members of these two subfamilies depolymerize microtubules via a similar mechanism [2,11–13]. However, unlike the well-studied kinesin-13 motor, MCAK, which does not translocate directionally and can depolymerize both ends of a microtubule, kinesin-8s translocate to and specifically act to destabilize plus-ends [11–13]. Furthermore, the rate of kinesin-8-dependent depolymerization is affected by the length of the microtubule substrate — kinesin-8 motors depolymerize longer microtubules faster than shorter ones *in vitro* [2,12]. This length-dependent effect on the depolymerization rate is likely due to the accumulation of more motor at the tips of longer microtubules [12]. Such a combination of activities could allow kinesin-8 motors in the context of a cell to specifically target depolymerizing activity to the plus-ends of unusually long or stable microtubules, such as kinetochore microtubules. In this manner, microtubule length and by extension, chromosome position would be self-limiting.

The length-dependent model could explain why the loss of kinesin-8 function in various experimental systems results in failed chromosome congression, abnormally long mitotic spindles and nuclear positioning defects, as all of these processes require regulation of microtubule length [12]. Consistent with this idea, the evidence presented by Mayr *et al.* [2] suggests that Kif18A exerts its effects on both spindle length and chromosome alignment by regulating the plus-end dynamics of kinetochore microtubules [2] (Figure 1A). Similarly, Kip3p regulates the plus-end dynamics of microtubules that interact with the cell cortex during nuclear positioning [11]. Taken together, these data suggest that kinesin-8 motors from yeast and human have similar biochemical activities that are utilized to directly regulate microtubule plus-end dynamics and microtubule length *in vivo*. Surprisingly, however, the specific effects of Kif18A and Kip3p on cellular microtubule dynamics are quite different.

Mayr *et al.* [2] report that Kif18A is needed to achieve normal rates of chromosome movement, as chromosome velocities are slower in its absence. In addition, the loss of Kif18A function results in spindle microtubules that are resistant to depolymerization, which leads the authors to conclude that microtubule dynamics in cells lacking Kif18A are suppressed [2]. Together, these results suggest a function for Kif18A in depolymerizing kinetochore microtubules to directly drive chromosome movements. These findings indicate that Kif18A has an important function in regulating kinetochore microtubule dynamics. But it is not entirely clear whether reduced chromosome velocity can account for the complete disruption of

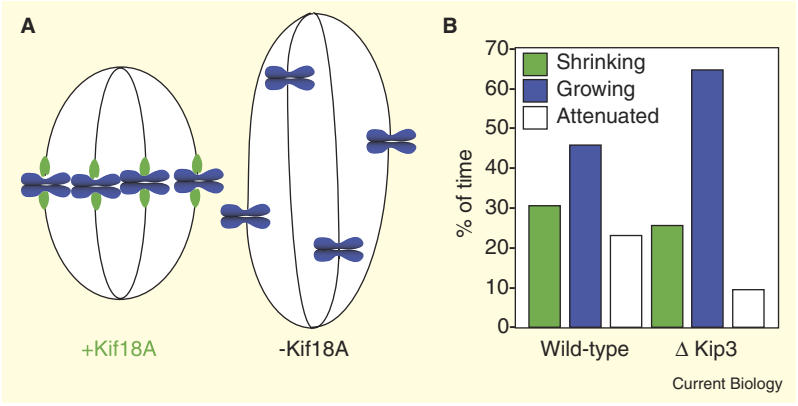

Figure 1. Effects of kinesin-8 depletion in live cells.

(A) Depletion of Kif18A results in longer spindles and chromosomes that do not line up at the metaphase plate. (B) Loss of yeast Kip3 results in microtubules that spend comparatively more time growing than attenuated relative to wild-type cells. Data modified from Gupta *et al.* [11].

congression seen in Kif18A-depleted cells. For example, tubulin mutations that suppress microtubule dynamics, and thus presumably reduce the rate of chromosome movement during mitosis, do not prevent proper chromosome positioning in yeast [14]. This raises a question about whether Kif18A might have additional functions during chromosome congression?

The measured effects of Kif18A on kinetochore microtubule dynamics correspond well with its ability to depolymerize stabilized microtubules *in vitro*, but they differ from the effects of *kip3* deletion on cytoplasmic microtubules in budding yeast, and uncovering the reasons for these differences will likely lead to a better understanding of kinesin-8 function. In a recent study, Gupta *et al.* [11] made detailed measurements of microtubule dynamic parameters in yeast cells lacking *kip3* (*kip3Δ* cells). They found that microtubules in *kip3Δ* cells spent less time in an attenuated state and more time growing or shrinking, indicating that microtubules are more dynamic in the absence of Kip3p (Figure 1B). In addition, the rate of microtubule depolymerization was significantly increased in the absence of Kip3p, suggesting that the motor might be needed to govern the velocity of depolymerization.

When considering the results of these two studies [2,11], it appears that the yeast kinesin-8 suppresses microtubule dynamics and reduces shortening velocity, while the human motor promotes dynamics and increases shortening velocity. The similar biochemical activities measured for Kif18A and Kip3p suggest that these differences are not likely due to intrinsic differences between the two motors. Are the differences an indication of motor regulation or indirect effects on other regulatory factors? Obviously, these questions warrant further investigation.

Mayr *et al.*'s [2] interesting study functionally dissects a novel component of the machinery for chromosome congression [2]. The complexity of the problem illuminated by their work and recent studies of Kip3p function [11,12,15] suggest that unraveling the molecular mechanisms that kinesin-8 family motors utilize to regulate dynamic microtubules will remain quite a fascinating problem for a number of years to come.

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Sex Determination: Controlling the Master

Sex is determined in *Drosophila* by the activity of the Sex-lethal master regulator. Activity of Sex-lethal is initiated early in females by chromosome-counting transcription factors, then reinforced by signaling through the Janus kinase pathway.

Douglas A. Harrison

Drosophila Sex-lethal (Sxl) is the prototype developmental 'master regulator', a term used to describe proteins whose activities are sufficient to initiate an entire developmental program. Sxl is the binary switch that determines whether a fly will develop as a male or a female [1]. But even the master is controlled. It has been known for some time that the on/off state of *Sxl* is decided by a set of early embryonic transcription factors, plus the Janus kinase (JAK) signaling pathway. But a recent *Current Biology* paper by Avila and